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Release of urokinase plasminogen activator receptor during urosepsis and endotoxemia

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Background. The urokinase receptor (uPAR; CD87) is a multifunctional molecule involved in fibrinolysis, in proteolysis, in renal tubular functions, and in migration and adhesion of inflammatory cells to the site of infection.

Methods. To gain insight into systemic and local release of uPAR and into its regulation during urosepsis, which is one of the leading causes of chronic renal failure, uPAR was measured in urine and plasma of healthy human controls ($N = 20$), patients with culture-proven urosepsis ($N = 30$), and healthy human volunteers intravenously injected with endotoxin ($N = 7$).

Results. Patients had elevated uPAR levels in both plasma and urine. Three hours after endotoxin challenge in volunteers, there was also a significant increase of uPAR in plasma and in urine. The urine/plasma ratio for uPAR was highly elevated during urosepsis and experimental endotoxemia, suggesting local production in the kidney. Accordingly, damaged tubuli strongly expressed uPAR during pyelonephritis. Moreover, tubular epithelial cells produced uPAR in vitro, and this secretion was strongly up-regulated after stimulation with interleukin-1β or tumor necrosis factor-α.

Conclusions. We found that uPAR is released systemically and in the urinary tract during urosepsis and experimental endotoxemia. This systemic and renal production of uPAR during pyelonephritis may play a central role in eliminating the infection and protecting renal function.

Urinary tract infections, most frequently caused by *Escherichia coli*, are one of the most important bacterial infections in females and in childhood [1]. Acute pyelonephritis is a frequent source of gram-negative sepsis and in 10 to 40% of cases leads to renal scarring. The course of urinary tract infection is determined by bacterial virulence factors and by host factors such as anatomical abnormalities of the urinary tract and defense mechanisms. Recently, attention has been paid to the role of various inflammatory pathways such as the local and systemic production of inflammatory cytokines and chemokines in the resistance to urinary tract infections [2–5]. A compensatory anti-inflammatory response generated at the systemic level takes also place during urosepsis and is characterized by the release of interleukin-10 (IL-10), soluble tumor necrosis factor (TNF) receptors, and IL-1 receptor antagonist [6].

Although urokinase type plasminogen activator (uPA) and its receptor (uPAR; CD87) were originally discovered in the urine as implied by the name, the role of uPAR has not been studied during urinary tract infections. uPAR is present at the cell surface of monocytes/macrophages [7, 8], neutrophils [9], T cells [10], endothelial cells [11], smooth muscle cells [12], and renal tubular epithelial cells [13]. Although uPAR is a glycosylphosphatidylinositol (GPI)-linked membrane protein and, therefore, lacks the transmembrane and cytoplasmatic sequences to induce signal transduction, uPAR can form complexes with CR3 (CD11b/CD18) on monocytes [14] and granulocytes [15], thus resulting in signal transduction. The fibrinolytic system consists of a number of proteases and protease inhibitors that regulate the generation of plasmin, the active end product of this pathway that dissolves fibrin clots. The formation of cell-associated plasmin is triggered by the binding of uPA to its receptor. Besides its essential role in fibrinolysis, the uPA/uPAR system also has an obligate function in chemotaxis of neutrophils and macrophages [16, 17]. Indeed, the uPA/uPAR system plays a role in adhesion and migration of neutrophils to the site of inflammation by an effect on β_2 integrins in vitro [9, 18–21]. Moreover, plasmin also induces proteolysis, which is a crucial step for cell migration. These data indicate that the activation

Key words: CD87, nephrotoxicity, urinary tract infection, tubular epithelial cells, cytokines, bacterial infection, anti-inflammatory response.

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of the uPA/uPAR system can initiate the inflammatory response, including chemotaxis and extravasation of inflammatory cells, necessary to clear the infection. In the urinary tract, the uPA/uPAR system might fulfill additional and essential functions. The uPA-catalyzed proteolytic activity can play an important role in the prevention of protein precipitation and subsequent tubular obstruction and preserve tubular function and integrity under physiological and pathological conditions [22]. Although the pathophysiological role of the uPA/uPAR system in renal function remains to be studied, an increasing number of studies correlate abnormalities in the fibrinolytic system with both acute and chronic renal diseases [23–26].

The present study sequentially measured plasma and urine concentrations of uPAR in patients with urosepsis during the first eight hours after the initiation of antibiotic treatment and in healthy volunteers intravenously injected with *E. coli* endotoxin. We also analyzed the expression of uPAR by immunohistochemistry on renal tissue of patients with pyelonephritis and its secretion in vitro by tubular epithelial cells.

METHODS

Patients with urosepsis and controls

Thirty patients (mean age ± SEM, 42 ± 3 years) with culture proven gram-negative urosepsis were studied. The present study in patients with urosepsis was performed simultaneously with an investigation determining the production of chemokines, the results of which have been published previously [27]. Patients older than 18 years of age, suspected of having gram-negative urosepsis and in whom antibiotic treatment was indicated, were eligible when they met the following criteria: acute symptoms of urinary tract infection, pyuria (leukocytes >10/hpf with epithelial cells <5/hpf), urine gram stain with gram-negative bacteria, and metabolic or hematologic signs of systemic infection, including two of the following three indicators: tachycardia (>90 beats/min), leukocytosis (>10,000/mm [3]) or fever (>38.0°C). Exclusion criteria were antibiotic use in the previous seven days, known hypersensitivity to any β-lactam antibiotics, a poor clinical condition, renal insufficiency (estimated creatinine clearance <30 mL/min), pregnancy or breast feeding, use of systemic corticosteroids or other immunosuppressive agents in the past three months, a history of seizures, use of any investigational drug within 30 days, or any clinically significant medical condition that would pose a risk to the patient should he/she participate. Patients received either a single dose of 1000 mg intravenous ceftazidime or a single dose of 500 mg intravenous imipemem, followed after eight hours by an antibiotic chosen by the clinician. Urine and heparinized blood were collected before the start of treatment (0 hour) and at two, four, and eight hours thereafter. The samples were centrifuged at 1500 × *g* for 20 minutes. Supernatants were collected and stored at −75°C until assays were performed. All patients fully recovered after treatment. Urine and heparinized blood were also collected from 20 healthy individuals, all of whom had sterile urine.

Experimental endotoxemia

In addition to the patients with urosepsis, seven healthy subjects (mean age ± SEM, 24 ± 1 years) were studied after intravenous administration of endotoxin [lipopolysaccharide (LPS)]. The subjects did not smoke, use any medication, or have a febrile illness in the month preceding the study. They were admitted to the clinical research unit at the Academic Medical Center after their medical history, physical examination, hematological and biochemical tests, chest x-ray, and echocardiogram had proved normal. Endotoxin (lipopolysaccharide, LPS standard lot G from *E. coli* obtained from the United Stated Pharmacopeia Convention Inc., Rockville, MD, USA) was given over one minute in an antecubital vein at a dose of 4 ng/kg body weight. Blood was collected by venapunctures directly before LPS administration (t = 0) and at t = 0.5, 1, 2, 3, 4, 5, 8, and 12 hours. Plasma was obtained by centrifuging at 1500 × *g* for 20 minutes. The urine was collected before LPS administration (t = 0) and at t = 3 and 6 hours.

The study was approved by the institutional scientific and ethics committees. Written informed consent was obtained from all patients and healthy subjects.

Human kidney biopsies

Human kidney samples were obtained either by percutaneous renal biopsies from patients with tubulointerstitial nephritis secondary to chronic pyelonephritis (N = 2) or from autopsy of patients with chronic pyelonephritis (N = 4). As control, we used a normal part of kidneys removed by nephrectomy for renal cell carcinoma (N = 5). All samples were frozen in liquid nitrogen without prefixation for immunohistochemical study.

Immunohistochemical study

Immunohistochemical studies were performed on cryostat tissue sections (4 μm). After fixation in acetone for 15 minutes at room temperature, slides were washed, preincubated first in 10% normal goat serum for 15 minutes, and then incubated for 16 hours at 4°C with an anti-human mouse IgG1 monoclonal anti-uPAR (CD87) R-phycoerythrin (PE)-conjugated antibody (Pharmingen, San Diego, CA, USA). The endogenous peroxidase activity was then blocked using 0.1% NaN_3 and 0.3% H_2O_2 in phosphate-buffered saline (PBS) for 15 minutes at room temperature. Sections were washed and incubated with polymerized horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG antibody (Powervision™;

Immunovision Techonology, Daly City, CA, USA). Enzyme activity of HRP was finally detected using 3-amino-9-ethyl-carbazole and counterstained with hematoxylin.

Cell culture

HK-2 cells, from the HPV 16-immortalized renal proximal tubular epithelial cell line obtained from normal adult human kidney, were purchased from ATCC (Rockville, MD, USA). Cells were grown to subconfluence in conditioned medium consisting of a 1:1 ratio of Dulbecco's modified Eagle's medium (DMEM; ICN, Costa Mesa, CA, USA) and Ham's F12 (DMEM/F12) medium (Life Technologies, Paisley, UK) supplemented with 5% heat-inactivated fetal calf serum (FCS; Life Technologies), 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mmol/L L-glutamine, 5 μg/mL insulin, 5 μg/mL transferrin, 5 ng/mL sodium selenite, 20 ng/mL tri-iodothyronine, 5 ng/mL hydrocortisone, 5 ng/mL PGE_1, and 5 ng/mL epidermal growth factor, all obtained from Sigma (St. Louis, MO, USA). For passage, confluent cells were harvested by trypsinization with 0.25% (wt/vol) trypsin-0.03% (wt/vol) ethylenediaminetetraacetic acid (EDTA) in PBS and plated again in conditioned DMEM/F12. For activation, cells were grown to confluency and were growth arrested in serum-free unconditioned medium for 24 hours, and all experiments were subsequently performed in unconditioned medium supplemented with 0.5% FCS. Cells at a concentration of 1×10^5 cells/mL were stimulated in 24-well plates with recombinant TNF-α (10 ng/mL) or recombinant IL-1β (10 ng/mL; Strathmann Biotech, GmbH, Hannover, Germany). Supernatants were harvested after 24, 48, and 72 hours of culture and were tested by enzyme-linked immunosorbent assay (ELISA) for uPAR production.

ELISA

Urokinase plasminogen activator receptor concentrations were measured by ELISA according to the recommendations of the manufacturer using purified monoclonal mouse antihuman uPAR (4 μg/mL; R&D Systems, Abingdon, UK) as coating antibody, biotinylated goat anti-human uPAR (100 ng/mL; R&D Systems) as detecting antibody, and recombinant human uPAR (R&D Systems) as the standard. The detection limit of the assay was 0.2 ng/mL. Urine concentrations are expressed as ng/mmol creatinine to correct for dilution.

Statistical analysis

Data are given as mean ± SEM when appropriate. Comparisons of uPAR concentrations were done by Mann-Whitney *U* test except for the analysis of the evolution of uPAR concentration in time during urosepsis and experimental endotoxemia where the one-way analysis of variance (ANOVA) was applied. Correlations between the uPAR concentration in urine and plasma as well as between the uPAR concentration and the APACHE II score were assessed by calculating the Spearman correlation. A probability of less than 0.05 was considered significant.

RESULTS

Clinical and microbiology data

The duration of symptoms before the first urine and plasma samples were taken was 2.9 ± 0.3 (mean ± SEM) days. *E. coli* was cultured form the urine of all except one patient. Ten patients had positive blood cultures, and all but one (*Proteus mirabilis*) were *E. coli.* The median APACHE II score on admission was six (range 0 to 17). All patients fully recovered after treatment.

uPAR secretion in plasma and in urine during urosepsis

The urokinase receptor uPAR was detectable at low levels in plasma (1.9 ± 0.4 ng/mL) and in urine (558 ± 66 ng/mmol creatinine) of healthy subjects. As shown in Figure 1, both plasma and urine levels of uPAR were higher in patients with urosepsis on admission (plasma, 21.1 ± 2.5 ng/mL, and urine, 18427 ± 5958 ng/mmol creatinine) than in healthy controls (both $P \leq 0.001$). The patients with positive blood cultures had a significantly higher concentration of uPAR in plasma but not in urine than patients with negative blood cultures (time of admission, 32.8 ± 4.6 vs. 16.3 ± 1.9 ng/mL, $P = 0.006$; 8 hours after admission, 35.6 ± 7.2 vs. 15.0 ± 2.2, $P = 0.002$). However, no significant correlation could be found between the individual APACHE II score and the levels of uPAR in plasma and urine. The type of antibiotic regimen did not significantly influence uPAR levels in urine or plasma (data not shown); therefore, both groups were pooled for further analysis. During the follow-up of eight hours, only urine levels of uPAR decreased (but not statistically significantly) eight hours after the initiation of antibiotic treatment. At the time of admission and four hours after, a significant correlation between uPAR concentrations in plasma and urine was found (0 hour, $r = 0.411$, $P = 0.041$; 4 hours, $r = 0.469$, $P = 0.012$).

uPAR secretion during experimental endotoxemia

To establish the role of the systemic inflammatory reaction in the up-regulation of uPAR in the circulation and in the urinary tract, plasma and urine levels of uPAR were measured at different time points after LPS injection in healthy humans. As shown in Figure 2A, LPS induced a significant increase in plasma uPAR concentration, with a peak plasma level three hours after challenge (5.2 ± 0.7 ng/mL, $P \leq 0.001$) to return to normal values after 12 hours (2.2 ± 0.2 ng/mL). Urine concentrations of uPAR increased within three hours after LPS

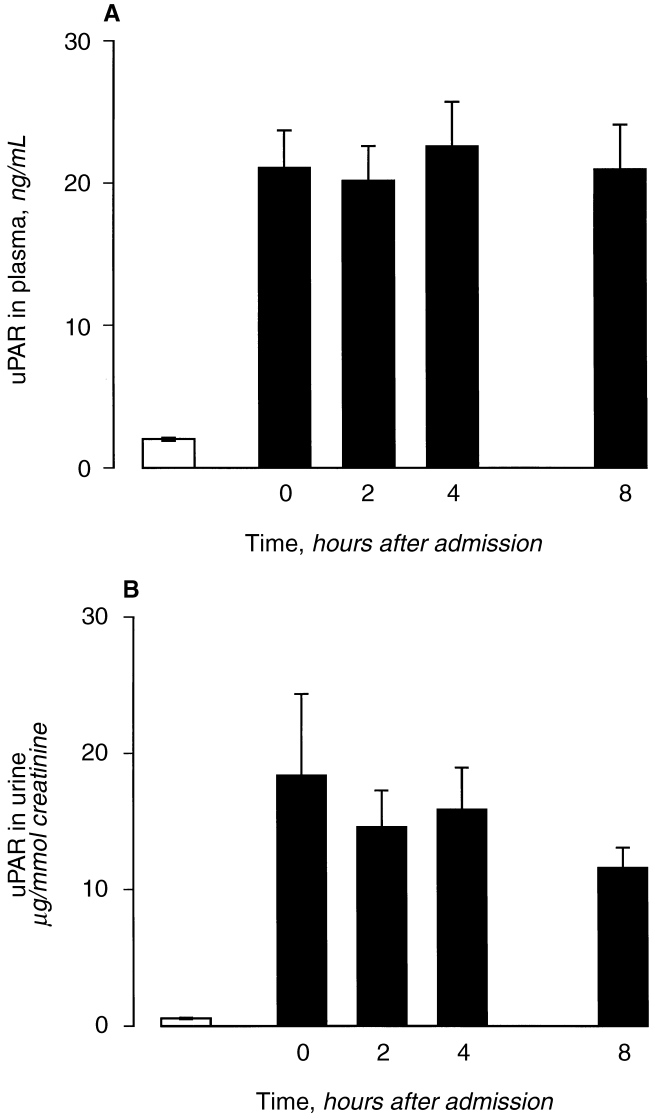

Fig. 1. Plasma (*A*) and urine (*B*) levels of urokinase plasminogen activator receptor (uPAR) in healthy subjects (□) and in patients with urosepsis (■). Samples from patients with urosepsis were collected on admission (t = 0) and at two, four, and eight hours after initiation of antibiotic therapy. Results are expressed as mean ± SEM. Patients with urosepsis ($N = 30$) had higher plasma and urine levels than controls ($N = 20$) throughout the follow-up (both $P < 0.001$ at all time points).

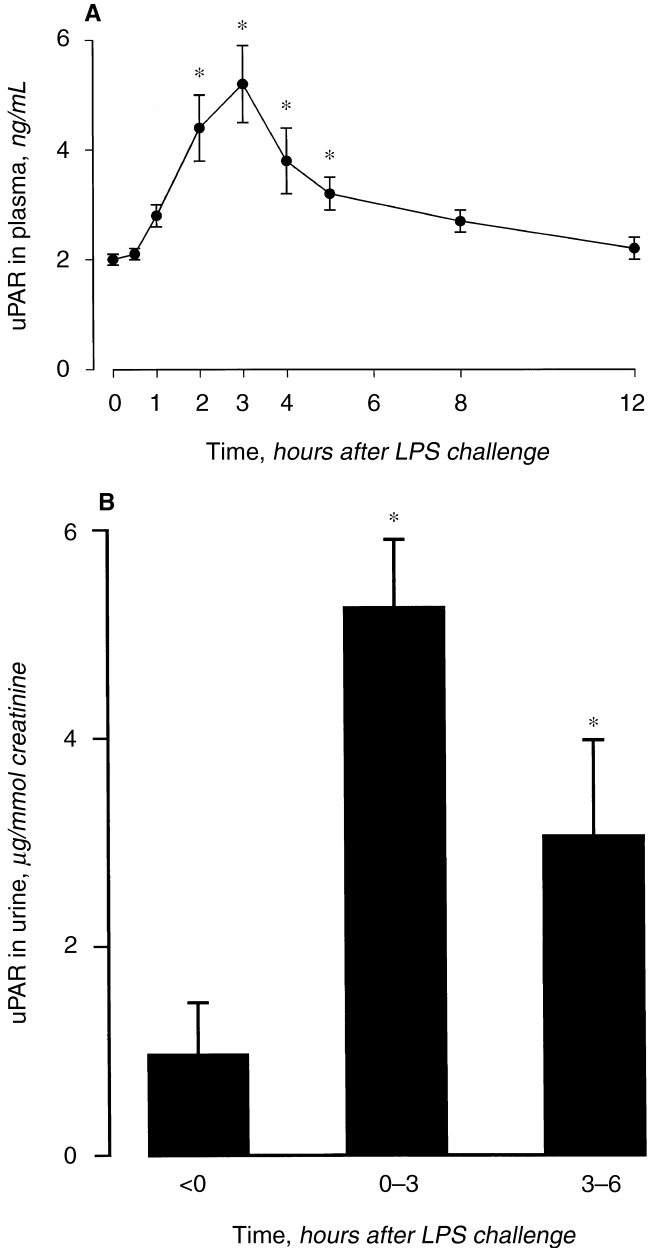

Fig. 2. Plasma and urine concentrations of uPAR during experimental endotoxemia. Plasma (*A*) and urine (*B*) concentrations of uPAR are expressed as mean ± SEM. Plasma concentrations of uPAR were measured prior to endotoxin injection (t = 0) and during a follow up of 12 hours in seven healthy volunteers. Urine concentrations were measured prior to endotoxin injection (t < 0) and in urine collected between zero and three hours after endotoxin administration (t = 0 to 3) and between three and six hours after endotoxin administration (t = 3 to 6). The asterisks indicate significant difference from the levels at t = 0 ($P < 0.005$).

exposure (Fig. 2B). No leukocytes were found in urine from any of the subjects injected with LPS at any time point. There was no significant correlation between the plasma and urine concentration of uPAR.

Renal production of uPAR during urosepsis and experimental endotoxemia

Having established that during urosepsis and experimental endotoxemia the secretion of uPAR both in plasma and in urine is significantly up-regulated, we sought to determine whether the kidney could be a major source of uPAR in both situations. As shown in Table 1, the ratio between the individual urine and plasma levels of uPAR was significantly higher at all time points during urosepsis compared with normal subjects. The urine/plasma ratio during experimental endotoxemia was also elevated, although the difference was not statis-

Table 1. Urine/plasma ratio of urokinase plasminogen activator receptor (uPAR) levels during urosepsis and experimental endotoxemia

Groups	Urine/plasma ratio of uPAR
Healthy subjects	433 ± 54
Urosepsis	
t = 0	972 ± 187[a]
t = 2	1147 ± 281[a]
t = 4	989 ± 152[a]
t = 8	1008 ± 187[a]
LPS volunteers	
t = 0	609 ± 401
t = 3	1014 ± 353
t = 6	1394 ± 387

Data are mean ± SEM of the individual ratio of urine and plasma concentration of uPAR in healthy subjects ($N = 20$), patients with urosepsis ($N = 30$) before initiation of antibiotic therapy (t = 0), and 2, 4 and 8 hours after, and healthy volunteers ($N = 7$) prior to endotoxin injection (t = 0), and 3 and 6 hours after the challenge.

[a] $P \leq 0.02$ compared with healthy subjects

tically significant. This high urine/plasma ratio suggests the participation of the kidney in the production of uPAR in the urine in both conditions. In order to confirm these findings, renal tissues obtained from patients suffering from chronic pyelonephritis were stained for uPAR by immunohistochemistry. As shown in Figure 3A, damaged tubuli were clearly positive for uPAR. In contrast, normal renal tissue did not show any positive staining (Fig. 3B).

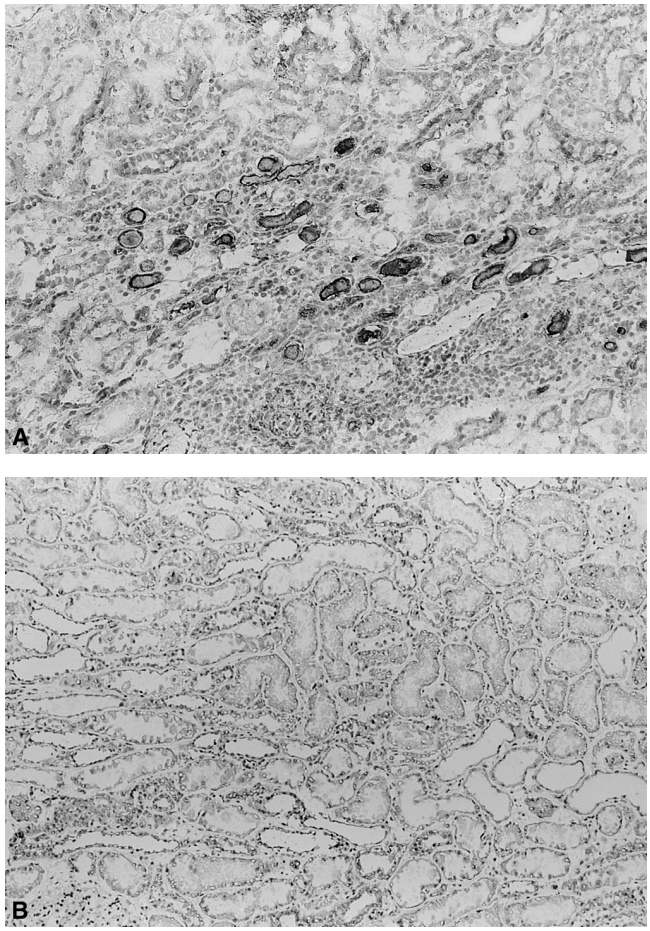

Fig. 3. Immunostaining for uPAR on renal tissue. Renal tissue obtained from patients with chronic pyelonephritis (*A*) and from normal part of kidneys removed by nephrectomy for renal cell carcinoma (*B*) were stained for uPAR. Damaged tubuli were clearly positive for uPAR in chronic pyelonephritis (A), while normal renal tissue did not show any positive staining (B; original magnification ×70).

Regulation of the production of uPAR by renal tubular epithelial cells in vitro

Having shown that uPAR is expressed on tubular epithelial cells during pyelonephritis and is secreted in large amounts in the urine during urosepsis and experimental endotoxemia, we studied the secretion of uPAR by renal tubular epithelial cells in vitro. As shown in Table 2, unstimulated proximal tubular epithelial cells produce small amounts of uPAR in vitro. After stimulation with IL-1β or TNF-α, the secretion of uPAR was significantly increased.

DISCUSSION

This study demonstrates the following: (*1*) soluble uPAR is elevated in plasma and urine during urosepsis and experimental endotoxemia; (*2*) uPAR is expressed by tubular epithelial cells during pyelonephritis; and (*3*) tubular epithelial cells stimulated with IL-1β or TNF-α produce large amounts of uPAR in vitro. Elevated soluble uPAR concentrations have been reported in the circulation of healthy volunteers after LPS administration [28], in patients with sepsis syndrome [29], with paroxysmal nocturnal hemoglobinuria [30], and with advanced malignancies [31, 32]. In the present study, we found high levels of soluble uPAR in both the plasma and the urine of LPS-exposed volunteers and of patients with urosepsis. Since different cell types are able to express uPAR, the cellular source of soluble uPAR in these patients and subjects exposed to LPS is probably multiple. As far as the systemic production is concerned, monocytes and granulocytes are likely candidates since the stimulation of human whole blood with endotoxin up-regulated the expression of uPAR on the surface of monocytes [28] and granulocytes (unpublished data).

The lack of correlation between urine and plasma levels of uPAR suggests that both systemic and renal production of uPAR take place during experimental endotoxemia. The large amounts of soluble uPAR found in the urine during urosepsis and experimental endotoxemia may reflect either its clearance from blood, its local production, or both. The relative high urine concentration of uPAR, the elevated urine/plasma ratio of uPAR during urosepsis and experimental endotoxemia, and the

Table 2. uPAR secretion by tubular epithelial cells in vitro

	uPAR (*ng/mL*) secretion after stimulation with[a]		
Stimulation during	0	IL-1β *10 ng/mL*	TNF-α *10 ng/mL*
24 hours	0.2 ± 0.1	0.8 ± 0.3[a]	0.6 ± 0.2[a]
48 hours	0.9 ± 0.2	1.9 ± 0.5[a]	1.4 ± 0.4[a]
72 hours	1.0 ± 0.3	2.3 ± 0.6[a]	1.6 ± 0.4

uPAR (ng/mL) secretion in supernatants of tubular epithelial cells unstimulated (0) and stimulated with IL-1β or TNF-α for 24, 48 and 72 hours. These results are expressed as mean ± SEM of 4 independent experiments.

[a] $P < 0.05$ compared to unstimulated cells

expression of uPAR on tubular epithelial cells in pyelonephritis show that a local production of uPAR occurs during renal inflammation. Recent evidence has highlighted that renal tubules are not only targets, but are also active participants in immune reactions. These cells exert crucial immunologic functions such as processing and presenting of foreign proteins [33] and secretion of proinflammatory cytokines such as IL-6 and TNF-α [34, 35], of chemokines [36–40] and of complement components (C3, C4, factor B) [41]. Different stimuli are able to activate tubular epithelial cells in vitro, among which are IL-1, IL-2, interferon-γ (IFN-γ), and the ligation of CD40 [42]. In the present study, we show a clear expression of uPAR on the surface of damaged tubular epithelial cells during pyelonephritis and a sustained secretion of uPAR by tubular epithelial cells in vitro after stimulation with IL-1β or TNF-α. To the best of our knowledge, there is only one study describing the distribution of uPAR in renal tissue in which uPAR expression was found in all segments of the tubular epithelium in normal renal tissue by immunohistochemistry [13]. Probably because of the use of other monoclonal antibodies, significant staining for uPAR was observed in only damaged tubuli during pyelonephritis. Along the same line, no expression for uPAR could be detected by FACS analysis on resting tubular epithelial cells (data not shown), but mRNA for uPAR was clearly present in unstimulated tubular epithelial cells by polymerase chain reaction analysis (data not shown). Altogether, these data suggest that tubular epithelial cells probably express a low level of uPAR under normal conditions and that this expression is up-regulated by inflammation. Accordingly, unstimulated tubular epithelial cells secrete small amounts of uPAR in vitro, and this secretion is increased by fourfold after stimulation with IL-1β and by threefold after stimulation with TNF-α. Both cytokines are known as powerful proinflammatory stimuli for tubular epithelial cells [42, 43]. These in vitro data suggest that the systemic release of inflammatory mediators (among which are IL-1 and TNF-α induced by LPS in vivo) can trigger the production of uPAR by tubular epithelial cells. This mechanism can explain the high urine/plasma ratio of uPAR during experimental endotoxemia. The regulation of uPAR expression on the surface of other cell types has been described. Briefly, IFN-γ, TNF-α, LPS, and urokinase collectively up-regulate the expression of uPAR on the surface of human monocytes, while granulocyte/macrophage-colony stimulating factor (GM-CSF) has no effect [7, 28]. Phorbol myrisate acetate (PMA), phytohemagglutinin (PHA), concanavalin A (Con A), IL-2, IL-4, and IL-7 induce the expression of uPAR at the surface of T cells. This induction is inhibited by transforming growth factor-β (TGF-β) [10]. As far as endothelial cells are concerned, PMA and basic fibroblast growth factor (FGF), but not TGF-β, can increase the production of uPAR [11, 44].

The exact function of soluble uPAR during infection remains to be elucidated. Soluble uPAR may facilitate the β_2 integrin-dependent adhesion of cells and/or vitronectin-mediated binding of uPA, especially when cells lack membrane-bound uPAR [18, 20, 21]. Soluble uPAR has been found to inhibit uPA binding to uPAR expressing cells, conceivably by competing with cell-associated uPAR for the binding of free uPA. The involvement of uPAR in leukocyte invasion through extracellular matrices is suggested by its expression on a variety of migratory cells and its polarization at the leading edge of migrating monocytes [45–47]. In vitro studies have further indicated that uPAR is crucial for chemotaxis of neutrophils and monocytes, suggesting that uPAR plays an important role in the orchestration of inflammatory reactions [9, 16, 47]. Little is known about uPAR regulation and function in vivo, but recently, it has been reported that uPAR-deficient mice have a defective β2-integrin–dependent migration of neutrophils into the peritoneal cavity after injection with thioglycollate [48] and an impaired neutrophil recruitment in response to pulmonary *Pseudomonas aeruginosa* infection [17]. Besides the systemic and local role of uPAR in fighting infection, the local expression and secretion of this receptor during renal inflammation may also fulfill an essential function in the maintenance of patency of the tubuli. Indeed, the binding of the zymogen pro-uPA to its receptor initiates an enzymatic cascade and induces the formation of plasmin, a trypsin-like protease. The presence of plasminogen activators is believed to be essential for the prevention of fibrin clot formation in both the kidney and the urine [13]. Tubular damage and tubulointerstitial fibrosis are frequent sequelae of pyelonephritis and contribute to the development chronic renal failure. Since the proteolytic activity of plasmin also plays a role in wound repair and matrix degradation [49], plasmin probably contributes to renal tissue remodeling by activation of latent metalloproteinases, which degrade extracellular matrix proteins in the kidney [50–52]. Future studies in animal models are needed to determine the exact function of uPAR in the clearance of infection, in the preservation of renal function, and in the prevention of renal scarring during pyelonephritis and urosepsis.

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APPENDIX

Abbreviations used in this article are: CD87, urokinase receptor; Con A, concanavalin A; DMEM, Dulbecco's modified Eagle's medium; EDTA, ethylediaminetetraacetic acid; FCS, fetal calf serum; FGF, fibroblast growth factor; GM-CSF, granulocyte/macrophage-colony stimulating factor; GPI, glycosylphosphatidylinositol; HRP, horseradish peroxidase; IL, interleukin; LPS, lipopolysaccharide; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PE, R-phycoerythrin; PHA, phytohemagglutinin; PMA, phorbol myrisate acetate; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; uPAR, urokinase receptor.

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